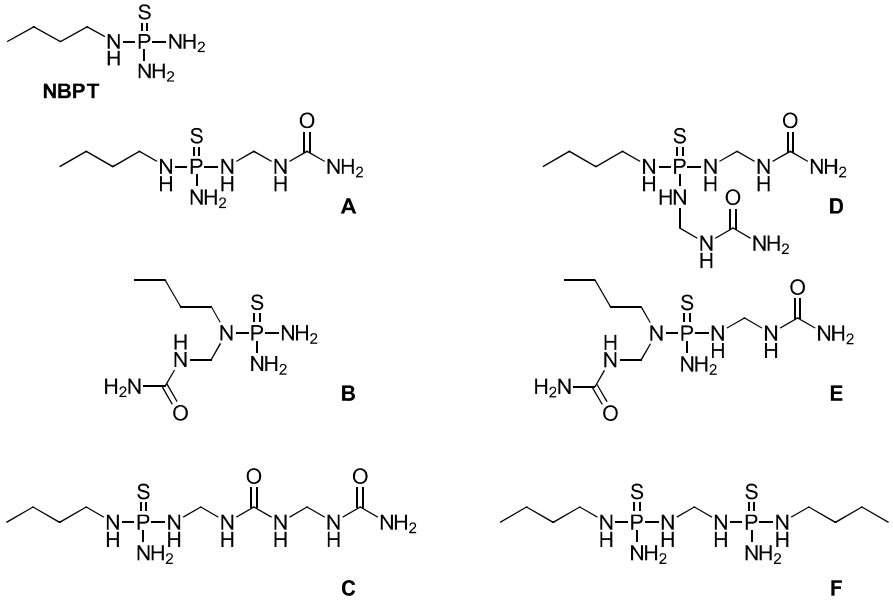
**Rationale for Read-across from NBPT to K32**

The following discussion describes the rationale and basis for using data on *N*-(*n*-butyl) thiophosphoric triamide (NBPT, Chemical Abstracts Index name: phosphorothioic triamide, N-butyl-, CAS RN 94317-64-3) to support a risk evaluation of K32. K32 is made from NBPT and is designed to hydrolyze to NBPT. The data conducted on K32 demonstrate that K32 does, in fact, hydrolyze to NBPT and the available toxicity information on K32 demonstrates the same endpoint of concern at comparable dose levels.

KAS reminds EPA that NBPT was the subject of a PMN, P-89-0538, and a TSCA Section 5(e) consent order. The PMN submitter developed and submitted data to EPA as required by the consent order, including the hydrolysis data discussed below. EPA subsequently revoked the consent order in 2002 based on the submitted data.

As described in the manufacturing section of this PMN, K32 is the reaction product of NBPT, urea (CAS RN 57-13-6) and formaldehyde (CAS RN 50-00-0). K32 is a mixture of isomers and oligomers as illustrated by the representative structures shown below.




Formaldehyde serves as a linking agent to combine NBPT via a methylene bridge with urea and itself. The ease of formation of these methylene bridges and the presence of multiple reaction sites on NBPT produces a complex reaction mixture. The multiple components in the K32 mixture represent multiple urea fragments added at different nitrogen moieties on the NBPT molecule. The actual number of urea fragments that may react with NBPT and/or the resulting adducts is indeterminate. Typically, a batch of K32 is made using equimolar quantities of NBPT, formaldehyde and urea, and will contain about 20% residual NBPT. Some of the higher oligomers are not soluble in water, but the majority of the expected adducts are highly soluble. Phosphorous content, which can be measured conveniently using ICP, can be used to quantitate the presence of NPBT and K32. Using this technique, >70% of the phosphorus content is present in solution after introduction of 50 mg/L of K32 in the water solubility study.

NBPT has been shown to be susceptible to hydrolysis under acid conditions. At pH 3, for example, the half-life of NBPT was determined to be 58 minutes.[1] Similarly, it is known that NBPT degrades more rapidly in acid soils. The efficiency of the urease inhibitory action of products containing the urea-formaldehyde-NBPT adducts (K32) is extended when compared to NBPT alone because the adducts must hydrolyze—to liberate NBPT— prior to the NBPT itself hydrolyzing. Inhibitory studies on K32 show it to be a very poor urease inhibitor unless there is concurrent hydrolysis to NBPT.

The rate of hydrolysis of K32 was explored at pH 4, pH 7, and pH 9, at $50^0$C. The profile at pH 4 shows almost complete hydrolysis of all K32 components and NBPT after the 5-day test. As expected, it is also apparent from the data that the rate of hydrolysis varies for the different components in K32. Structural features that would be particularly susceptible to hydrolysis under acid conditions include the methylene-urea ($CH_2$-N-C=O) and methylene-NBPT ($CH_2$-N-P=S) bonds. At pH 7, most components show no change in concentration after 5 days. There are, however, a small number of components that show a notable change in concentration after 5 days. One of these components, likely one of the higher oligomers based on LC-MS analysis, hydrolyzes and shows a decline in concentration. Conversely, the second component that changes is NBPT, which shows an increase in concentration, as might be expected. It is clear to KAS that one of the adducts releases NBPT, which is stable at pH 7 (with a hydrolysis half-life of 92 days) and consequently its relative concentration increases. From the labile groups identified as susceptible to hydrolysis, the methylene-urea substituents would appear to be the most likely to hydrolyze at pH 7. The hydrolysis profile at pH 9 is similar to the one observed at pH 7. Hydrolysis of a K32 component, as evidenced by its declining concentration, is concurrent with an increase in the concentration of NBPT.

We also note that the design of NBPT-methylene urea adducts in K32 mirrors the same chemical moiety present in urea-formaldehyde (UF), slow-release fertilizers. UF fertilizers are known to degrade via hydrolysis of the $CH_2$-N-C=O moiety, which results from the condensation of urea and formaldehyde.[2] This moiety also is present in K32.

---

1 PTRL West (1996) Hydrolysis of [$^{14}$C]-NBPT at a function of pH at 25°C, PTRL Report No. 483W-1, PTRL West Inc, USA (unpublished study).

2 Masahito Hayatsu. A Novel Function of Controlled-Release Nitrogen Fertilizers. Microbes Environ., 2014 June; 29(2): 121-122. Published online 2014 Jul 19, doi: 10.1264/jsme2.ME2902rh. PMCID: PMC4103517.

The toxicity of NBPT centers on or is driven by the thiophosphoric acid triamide. Similarly, the only recognized structural alert in K32 is the thiophosphoric acid amides.[3] The addition to NBPT of the methylene-bridged urea substituents changes the pharmacokinetics of the adducts, but does not alter the basic premise that observed toxicity will be dominated by the thiophosphoric acid triamide moiety.

The hydrolysis characteristics of K32 as described above support the argument that it is appropriate to read-across from NBPT; under environmental or physiological conditions where hydrolysis can occur, K32 will transform into NBPT. In fact, because the K32 mixture is essentially a slow-release form of NBPT, the use of toxicity information from the latter to characterize the former may be considered as the more conservative approach. The read-across assumes that the NBPT-containing components in K32 hydrolyze to release NBPT in the organism in question.

The closest available comparison in terms of route of administration and study duration between NBPT and K32 is a 15-day Range Finding study (NBPT) and a 28-day Repeated Dose study (K32) conducted in the rat by oral (gavage) administration. In the range finding study, NBPT was administered to Sprague Dawley rats (5/sex) at 0, 250, 500, 1000 and 2000 mg/kg bw/d by gavage for 15 days. No death occurred during the treatment phase of the study. At the top two dose levels, animals exhibited salivation and languid behavior. Clinical chemistry results showed significant decrease in blood urea nitrogen (BUN) in all dosed animals except the lowest dose males, significantly decreased total cholesterol in the highest dose groups, and significantly decreased triglycerides in highest dose males. An increase of alanine aminotransferase (ALT) was seen in the two mid-dose male groups. At necropsy, liver weights were not affected by the treatment, but the absolute and relative spleen weights were reduced in the highest dose animals. The study included an assay on cholinesterase activities. The assay showed that the cholinesterase activities of erythrocyte and brain were significantly inhibited by NBPT in a dose-dependent manner at the top 3 and top 2 dose levels respectively.

Dose levels in the 28-day repeated dose study with K32 were set at 0, 250, 500, and 1000 mg/kg. At the conclusion of the study, there were no test-substance related mortalities or changes in functional observation battery, motor activity, body weight or body weight gain, minimal changes in clinical chemistry, and no macroscopic, microscopic or organ weight changes. Preliminary information on K32 showed reduced red blood cells (RBC) and brain cholinesterase activity at the highest dose (1000 mg/kg) consistent with observed cholinesterase inhibition in the 15-day study with NBPT. Although the lower doses were not reviewed in the initial 28-day study, a repeat study is underway and will be submitted to EPA when complete.

The effects associated with K32 in test animals are generally indicative of lower toxicity at comparable dose levels with NBPT. The results above suggest a similar mode of action

---

[3] For a compilation of structural alerts, *see* U.S. EPA, *Sustainable Futures / P2 Framework Manual 2012*, , available at https://www.epa.gov/sustainable-futures/sustainable-futures-p2-framework-manual

for NBPT and K32 where interaction of the thiophosphoryl group with cellular components becomes a key initiating event. As observed in both NBPT and K32, and in keeping with similar thiophosphoryl chemistries, the primary endpoint of concern is cholinesterase inhibition. This toxicity profile is consistent with the anticipated slow release of NBPT as a modulator in the expression of systemic toxicity by K32.